

Interrelationships between *Acremonium lolii*, Peramine, and Lolitrem B in Perennial Ryegrass

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Perennial ryegrass (*Lolium perenne* L.) is commonly infected with the endophytic fungus *Acremonium lolii* in a mutualistic relationship. The fungus produces a number of alkaloids, some of which are responsible for causing livestock disorders and/or for conferring insect resistance to the host grass. Little is known about the interrelationship between fungal growth and alkaloid production in the ryegrass plant and how this varies throughout the year. The concentrations of *A. lolii* and two of its alkaloid metabolites, lolitrem B and peramine, were monitored in basal (mainly leaf sheath) and upper (mainly leaf blade) parts of 17 endophyte-infected ryegrass plants on a monthly basis for 1 year. *A. lolii*, lolitrem B, and peramine concentrations were lowest in winter. The highest *A. lolii* concentrations were recorded in early summer, which coincided with the development of plant reproductive structures. Lolitrem B concentrations were highest from summer to early autumn and were consistently highest in the basal part of the plant. Peramine concentrations were generally highest in the upper part of the plant. Individual plants contained different levels of *A. lolii*, lolitrem B and peramine. These differences were generally maintained throughout the year. Although data for each month were variable, regression analyses showed that yearly mean concentrations of lolitrem B and peramine in individual plants were closely related to, and therefore probably largely determined by, yearly mean concentrations of *A. lolii*.

Perennial ryegrass (*Lolium perenne* L.) is often infected with the clavicipitaceous endophytic fungus *Acremonium lolii* Latch, Christensen, and Samuels. Although its presence was known as early as the turn of the century (29), the biological significance of *A. lolii* in perennial ryegrass was only revealed in the early 1980s following two major discoveries. The first discovery established a link between the presence of *A. lolii* in perennial ryegrass and "ryegrass staggers," a neurological disorder sometimes seen in livestock grazing on ryegrass-dominant pastures (27). The second discovery established a connection between the presence of *A. lolii* and resistance of perennial ryegrass to Argentine stem weevil (*Listronotus bonariensis* (Kuschel)) (Coleoptera: Curculionidae) (46). Argentine stem weevil is ranked as the most important pasture insect pest in New Zealand, the total cost to the country being estimated at \$78 to 251 million per year (44). Thus, in terms of its effects on Argentine stem weevil and livestock, *A. lolii* has, respectively, both desirable and undesirable attributes.

A number of alkaloids are produced by *A. lolii* in association with the ryegrass. It was found that ryegrass staggers was caused largely by the presence of lolitrem B (32), while resistance to Argentine stem weevil was due principally to the presence of peramine (48). Hence, it was considered possible, through selection or genetic manipulation, to develop a ryegrass-*A. lolii* association which did not cause ryegrass staggers in livestock but which retained resistance to the Argentine stem weevil. However, since these initial discoveries, the picture has become considerably more complex. For example, with the improvement in technology, the number of alkaloids found in *A. lolii*-infected perennial ryegrass has increased dramatically (37, 49). Also, three more pasture-dwelling insect species in New Zealand, the black beetle (*Heteronychus arator* (F.)) (6), the cutworm (*Graphania mutans* Walker) (21), and

the pasture mealy bug (*Balanococcus poae* (Maskell)) (43), have been found to be adversely affected by the presence of *A. lolii* in perennial ryegrass. At least in the case of the black beetle, alkaloids other than peramine are responsible for this resistance (5, 7). In addition, livestock disorders other than ryegrass staggers, such as endocrine imbalance (26), heat stress (25), and reduced live-weight gains (23, 24, 26), have been reported for sheep grazing on *A. lolii*-infected ryegrass.

In order to resolve many of these complications, there is a great need for more information on the biology of the perennial ryegrass-*A. lolii* association. Two important aspects of the association are (i) the growth of the endophyte within the host grass and (ii) the production of alkaloids and how these vary throughout the year. Both enzyme-linked immunosorbent assay (ELISA) technology (23, 24) and counting of fungal hyphae within grass material (19, 20, 38) have been used previously to investigate seasonal changes of *A. lolii* concentration in infected perennial ryegrass. Seasonal lolitrem B concentrations in perennial ryegrass have also been monitored previously by using high-performance liquid chromatography (HPLC) (19, 45). However, in all of these studies *A. lolii* and lolitrem B concentrations were monitored by taking samples from pastures which, over time, were not derived from the same plants. This could have introduced some unnecessary variability into the data. Also, in the studies in which ELISA was used, *A. lolii* concentrations were not monitored over a full 12-month period, since the aim was to examine the seasonality of *A. lolii* with respect to the occurrence of ryegrass staggers (i.e., spring [September] to autumn [April]). Finally, in previous studies, with the exception of the investigation by di Menna et al. (19), who measured lolitrem B, alkaloid concentrations were not monitored simultaneously with *A. lolii* concentrations. The purpose of the present study was therefore to examine the interrelationships between *A. lolii*, lolitrem B, and peramine by sampling a number of individual *A. lolii*-infected plants throughout the year. Some preliminary results for the period from January 1990 to December 1990 have been reported elsewhere (8).

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MATERIALS AND METHODS

Plant maintenance and sampling. The 17 *A. lolii*-infected perennial ryegrass plants used in the study originated from a number of hill country pastures in the North Island of New Zealand (52). The plants selected encompassed a wide range of lolitrem B and *A. lolii* concentrations and were taken from pastures which were at least 20 years old. Plants were grown outdoors at the Ruakura Agricultural Research Centre, Hamilton, New Zealand (latitude, 37°47' south; longitude, 175°19' east; altitude, 40 m above sea level) at approximately 50-cm interplant spacings in soil which was kept weed free by hand weeding. Between December 1991 and November 1992, the plants were sampled on a monthly basis on the 20th day of each month. At each sampling, two plant components were harvested. Trimming each plant to a height of 8 cm from ground level yielded the regrowth component. Throughout most of the year, this consisted of leaf blade material only. However, during spring and summer (October to January), the regrowth component also contained parts of developing reproductive tillers (nodes, internodes, and inflorescences). Samples of approximately 20 to 30 tillers were then taken from each plant by harvesting at ground level with a scalpel. This yielded the basal component, which consisted mainly of leaf sheath and lower leaf blade material. The basal and regrowth components from each plant were washed in cold tap water to remove soil debris before being placed in separate plastic bags and immediately frozen at -20°C. The samples were then freeze-dried, finely ground in an Ika model MFC Culatti micro-hammer mill and stored at -20°C before being analyzed for *A. lolii*, peramine, and lolitrem B contents.

***A. lolii* analysis.** Perennial ryegrass samples were analyzed for *A. lolii* by ELISA.

ELISA development. A polyclonal double-antibody sandwich ELISA based on the method of Clark and Adams (17) was employed. The coating and disclosing antibodies were raised in two different rabbits which were inoculated with two different forms of *A. lolii* antigens. It was found that this combination of antibodies considerably reduced nonspecific binding compared with other antibody combinations (4). The coating antibody was derived from a rabbit inoculated with *A. lolii* antigens purified from the broth of a cultured endophyte (soluble-antigen fraction), while the disclosing antibody was raised in a rabbit injected with the mycelial-antigen fraction. The mycelial-antigen fraction was prepared as follows. *A. lolii* mycelium was isolated from perennial ryegrass seed (cv. Ellett) by the method of Neill (42) and was cultured in glucose (2%)–peptone (1%)–yeast extract (0.5%) broth. The mycelium was then filtered, resuspended in phosphate-buffered saline (PBS) at a concentration of 50 mg/ml, and homogenized. A procedure similar to that described by Musgrave et al. (41) was used to prepare the soluble-antigen fraction of *A. lolii*. Absolute ethanol (2.5 volumes) was added dropwise to measured volumes of the *A. lolii* broth supernatant. After the suspension was stored at -20°C for 16 h, the precipitate was collected by centrifugation at 10,400 × g for 20 min at 4°C. The pellet was resuspended in 2 ml of half-strength PBS and dialyzed against two changes of 2 liters of half-strength PBS at 4°C for 24 h. The dialysate was stored at a concentration of 1 mg/ml at -20°C ready for injection.

Each rabbit was injected subcutaneously with 2 ml of antigen emulsified with 1.25 ml of Freund's adjuvant three times, with 14 days between injections. Freund's complete adjuvant was used for the first injection, while Freund's incomplete adjuvant was used for the second and third injections. Rabbits were bled by nicking the marginal ear vein 7 to 12 days after the final booster. Blood samples were incubated overnight at 4°C, after which they were clarified by low-speed centrifugation (1,000 × g for 10 min) and semipurified by ammonium sulfate precipitation and ion exchange chromatography with DEAE cellulose (as described by Musgrave [39]). The enzyme label used was alkaline phosphatase. Enzyme-antibody conjugates were prepared by the method used by Musgrave (39).

Grass sample preparation and ELISA analysis. Grass samples were freeze-dried, since it was found that oven drying at 60°C reduced absorbances in *A. lolii*-infected samples to such an extent that the ELISA could no longer consistently distinguish *A. lolii*-infected from *A. lolii*-free grass samples (4). Grass samples were extracted for 6 h at room temperature at a concentration of 20 mg/ml in PBS–polyvinylpyrrolidone (2%, wt/vol)–polyoxyethylene (20)–sorbitan monolaurate (Tween 20) (0.5%, vol/vol). Three replicate wells per grass sample were used. To minimize the effects of interplate variation, each grass sample was analyzed twice for *A. lolii*. In each analysis, ELISA plates contained only the samples which were to be directly compared. In the first analysis, all plant material harvested in the same month was analyzed on the same ELISA plate, enabling accurate interplant comparisons to be made. In the second analysis, all plant material harvested from the same plant during the entire year was analyzed on the same ELISA plate so that the seasonality of *A. lolii* could be accurately determined. Extracts from *A. lolii*-free control plants were included on all plates. Also included were standards containing a 0.1-mg/ml *A. lolii* solution. This procedure enabled *A. lolii* concentrations to be calculated from an *A. lolii* dilution series included on a separate plate. The limit of detection of the assay was approximately 0.01 µg/ml (4).

The coating antibody concentration was 1.4 µg/ml, and the conjugate antibody concentration was 1.0 µg/ml. Following the antibody-coating step (6 h at 27°C), the plates were blocked with a 0.7% bovine serum albumin solution (1 h at 27°C). Grass sample incubation (16 h at 4°C) was followed by the conjugate incubation (7 h at 27°C) and finally the substrate incubation (90 min at room temperature).

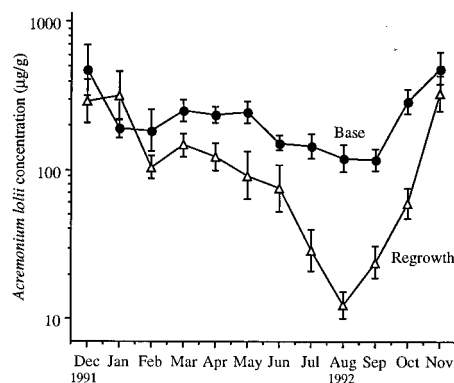


FIG. 1. Changes in mean monthly *A. lolii* concentrations in basal and regrowth components of infected perennial ryegrass plants during 1991 and 1992. Vertical bars represent the standard error of the mean ($n = 17$).

The substrate used was *p*-nitrophenyl phosphate at 1.0 mg/ml. A_{405} was measured with a Bio-Rad model 3550 microplate reader. The plates were washed three times with PBS after the antibody-coating and -blocking steps and twice with PBS-Tween (0.05%) and once with PBS after the sample and conjugate incubation steps.

Lolitrem B analysis. Lolitrem B was analyzed by a modification of the HPLC method of Gallagher et al. (30). One hundred-milligram samples of finely ground freeze-dried grass were weighed in 1.5-ml Eppendorf centrifuge tubes before extraction in 1.0 ml of chloroform-methanol (2:1) for 1 h on an orbital shaker. The tubes were then centrifuged for 5 min at 12,000 rpm in a Heraeus Christ Biofuge A centrifuge, and 0.5-ml aliquots of the extraction solvent were dried under nitrogen before being resuspended in 0.5 ml of dichloromethane. Sep-pak silica cartridges (Waters) were used for the sample cleanup, as described by Gallagher et al. (30). The HPLC system consisted of a Waters M-45 pump and a Waters 470 scanning fluorescence detector with an excitation wavelength of 265 nm and an emission wavelength of 440 nm. Other details of the HPLC method are as described by Gallagher et al. (30). The limit of detection of the assay was 0.01 µg/g.

Peramine analysis. Peramine was analyzed by a modification of a method supplied by B. A. Tapper (AgResearch, Palmerston North, New Zealand). This method differed considerably from that reported by Tapper et al. (51). Finely ground freeze-dried grass material was extracted (20 mg/ml) in 30% propan-2-ol for 30 min at 90°C. Ten microliters of a homoperamine internal standard solution (100 µg/ml) was added to the grass prior to extraction. Peramine was removed from grass extracts by passing 1-ml portions through preconditioned 1-ml Varian Bond Elut carboxylic acid (CBA) columns packed with 100 mg of adsorbent. The peramine was then eluted with 600 µl of a 5% formic acid–40% methanol solution. Peramine was measured by HPLC with a Spectra-Physics (model 740) liquid chromatograph fitted with an Alltech Econosphere silica column (5-µm particle size; 150 by 4.6 mm). Detection was performed with a Shimadzu variable wavelength spectrophotometric detector set at 230 nm. The quantity of peramine in 20-µl injection samples was determined by comparing the peak heights with those of peramine (1 µg/ml) in a peramine (1 µg/ml) and homoperamine (1 µg/ml) mixed standard. The limit of peramine detection was approximately 0.5 µg/g.

Statistical analysis. The mean *A. lolii*, peramine, and lolitrem B concentrations plus the standard errors of the means were calculated for each month. *A. lolii*, peramine, and lolitrem B concentration changes over time were compared by paired-difference *t* tests. Linear regression analysis was used to determine the relationships between *A. lolii*, peramine, and lolitrem B concentrations in individual plants.

RESULTS

In the basal component of the plant, mean *A. lolii* concentrations were highest in late spring and early summer (December 1991 and October and November 1992) and lowest in winter and early spring (June to September 1992) (Fig. 1).

The seasonal pattern of mean *A. lolii* concentrations in the regrowth component was similar to that observed for the basal component, with the highest concentrations being recorded in spring and summer (December 1991, January 1992, and November 1992) and the lowest being recorded in winter and early spring (July to September 1992) (Fig. 1). Mean *A. lolii* concentrations in the regrowth component were lower than

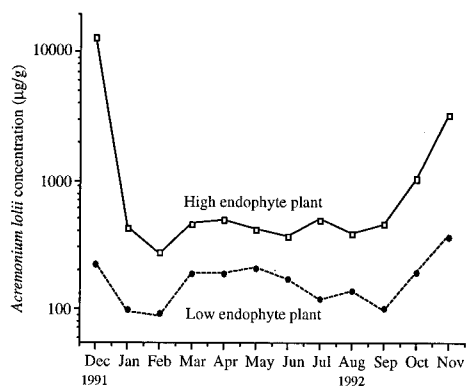


FIG. 2. Changes in *A. lolii* concentrations in the basal component of representative perennial ryegrass plants with high and low endophyte concentrations.

those in the basal component for much of the year, although they were similar during the summer (December 1991, January 1992, and November 1992).

Interplant comparisons revealed that within each month, individual plants contained different concentrations of *A. lolii*. These differences in *A. lolii* concentration were relatively constant throughout the year. Thus, it was generally possible to characterize plants on the basis of *A. lolii* content (Fig. 2).

Lolitre B concentrations in the basal component peaked during summer and early autumn (January to April 1992) and declined to a relatively stable low level during winter and spring (August to November 1992) (Fig. 3). The highest mean lolitre B concentration in the basal component for the year (4.62 µg/g) was recorded in January 1992, while the lowest (0.21 µg/g) was recorded in October 1992.

Lolitre B concentrations in the regrowth component were lower than those in the basal component for all harvests, but the seasonal pattern was similar to that of the basal component (Fig. 3). Lolitre B concentrations in the regrowth component were highest during summer and early autumn (December 1991 to April 1992) and lowest during winter and early spring (May to November 1992). The highest mean lolitre B concentration in the regrowth component (1.67 µg/g) was recorded in January, while the lowest (0.03 µg/g) was recorded in July.

In January 1992 (summer) and July 1992 (winter), lolitre B levels in individual plants ranged from 1.76 to 9.77 µg/g and

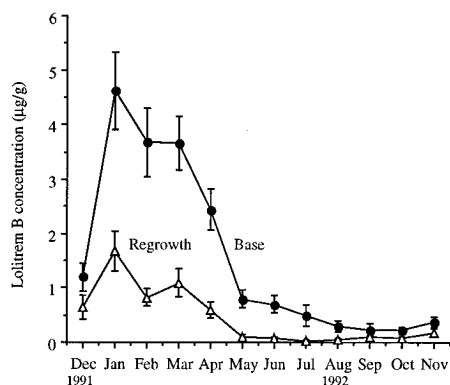


FIG. 3. Changes in mean monthly lolitre B concentrations in basal and regrowth components of *A. lolii*-infected perennial ryegrass plants during 1991 and 1992. Vertical bars represent the standard error of the mean ($n = 17$).

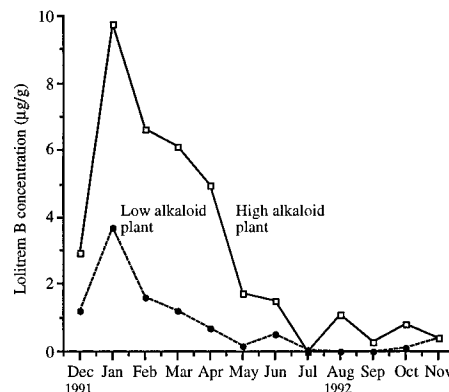


FIG. 4. Changes in lolitre B concentrations in the basal component of representative *A. lolii*-infected perennial ryegrass plants with high and low alkaloid concentrations.

from nondetectable to 2.59 µg/g, respectively, in the basal component and from 0.28 to 5.24 µg/g and from nondetectable to 0.12 µg/g, respectively, in the regrowth component. Thus, within each month, individual plants usually contained different concentrations of lolitre B. These differences were generally maintained throughout the year. It was therefore possible to identify associations producing high and low levels of lolitre B (Fig. 4), particularly during the summer-autumn (January 1992 to April 1992) period when the differences were greatest (Fig. 3 and 4).

Mean peramine concentrations in the basal component were relatively stable during summer and autumn (December 1991 to May 1992) and ranged from 14.5 to 18.1 µg/g (Fig. 5). Peramine concentrations declined during winter and reached their lowest level of the year (5.5 µg/g) in August. After August, peramine concentrations in the basal component increased steadily and, by October, were nearly as high as those recorded from January to May 1992.

For most months of the year, peramine concentrations in the regrowth component were higher than those in the basal component (Fig. 5). The highest mean peramine concentrations were recorded between late summer and early winter (February to July 1992) (23.5 to 34.2 µg/g), while the lowest were recorded in winter and early spring (August to October 1992) (8.6 to 11.7 µg/g).

January and July 1992 levels of peramine in individual plants

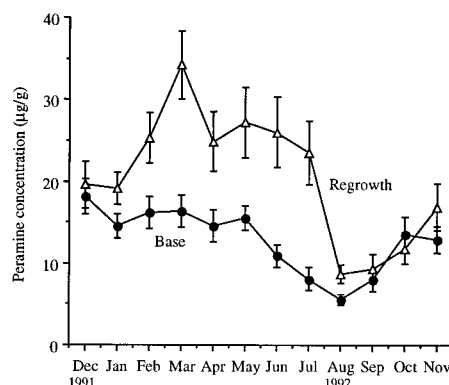


FIG. 5. Changes in mean monthly peramine concentrations in basal and regrowth components of *A. lolii*-infected perennial ryegrass plants during 1991 and 1992. Vertical bars represent the standard error of the mean ($n = 17$).

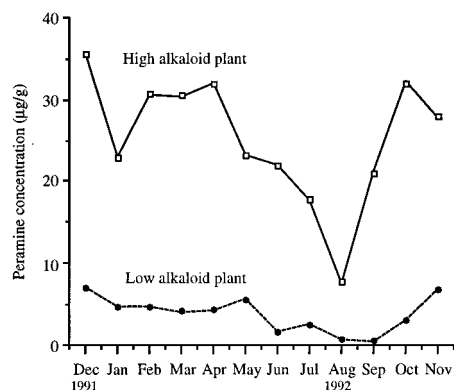


FIG. 6. Changes in peramine concentrations in the basal component of representative *A. lolii*-infected perennial ryegrass plants with high and low alkaloid concentrations.

ranged from 4.8 to 23.0 µg/g and from 1.7 to 17.7 µg/g, respectively, in the basal component and from 5.3 to 35.0 µg/g and from 7.0 to 52.5 µg/g, respectively, in the regrowth component. As with *A. lolii* and lolitrem B, differences in the concentrations of peramine in individual ryegrass plants were generally maintained throughout the year, enabling the identification of associations which produced high or low levels of peramine (Fig. 6).

Lolitrems B concentrations in individual plants were significantly positively correlated with peramine concentrations in 3 of 12 (R^2 values ranged from 0.0 to 58.5) and 6 of 12 (R^2 values ranged from 0.0 to 51.2) months for basal and regrowth components, respectively. Also, yearly mean lolitrem B concentrations in both basal and regrowth components of individual plants were significantly ($P < 0.01$ and $P < 0.001$, respectively) positively correlated with yearly mean peramine concentrations (Fig. 7).

Lolitrems B concentrations in individual plants were significantly positively correlated with *A. lolii* concentrations on 4 of 12 (R^2 values ranged from 0.0 to 42.0) and 5 of 12 (R^2 values ranged from -5.3 to 52.0) sampling occasions in the basal and regrowth components, respectively, while peramine concentrations were significantly positively correlated with *A. lolii* concentrations for 11 of 12 (R^2 values ranged from 0.0 to 86.3) and 7 of 12 (R^2 values ranged from 0.0 to 76.6) months in the basal and regrowth components, respectively. Yearly mean lolitrem

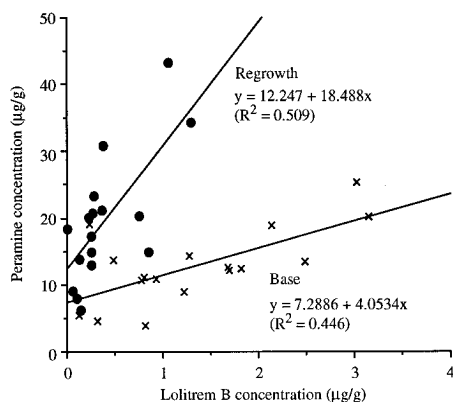


FIG. 7. Correlations between yearly mean lolitrem B and peramine concentrations in basal and regrowth components of individual *A. lolii*-infected perennial ryegrass plants.

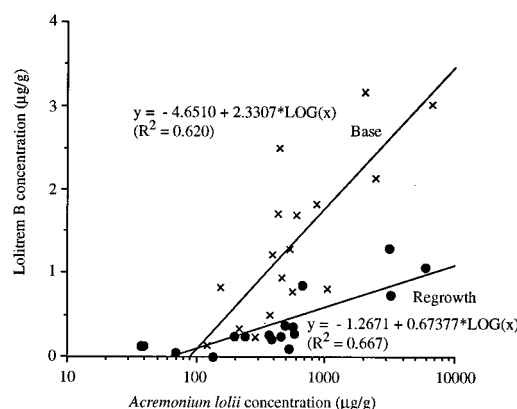


FIG. 8. Correlations between yearly mean *A. lolii* and lolitrem B concentrations in basal and regrowth components of individual perennial ryegrass plants.

B (Fig. 8) and peramine (Fig. 9) concentrations in basal and regrowth components of individual plants were significantly ($P < 0.001$) positively correlated with yearly mean *A. lolii* concentrations.

Yearly mean lolitrem B and peramine concentrations were significantly ($P < 0.001$) positively correlated with yearly mean lolitrem B and peramine concentrations (R^2 values of 86.5 and 84.5, respectively) in the same plants sampled in 1990 (8).

DISCUSSION

The highest and lowest mean *A. lolii* concentrations were recorded during late spring-early summer and winter-early spring, respectively (Fig. 1). Similar seasonal changes in *A. lolii* concentrations were recorded by Fletcher (23, 24) in Canterbury, New Zealand, using ELISA and Mortimer et al. (38), di Menna and Waller (20), and di Menna et al. (19) in the central North Island and Waikato, New Zealand, using the hyphal-count method. These similar results were obtained despite the fact that the methods used to estimate *A. lolii* concentrations, the nature of the test samples, and the time and place in which plant material was harvested differed widely. It is apparent therefore that *A. lolii* concentrations in perennial ryegrass change with time and that the seasonal nature of this change is generally consistent from year to year. This observation suggests that physical environmental factors which undergo similar seasonal changes, such as temperature, photoperiod, and

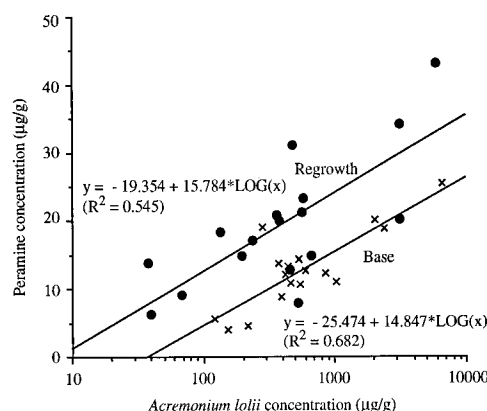


FIG. 9. Correlations between yearly mean *A. lolii* and peramine concentrations in basal and regrowth components of individual perennial ryegrass plants.

rainfall, are likely to be important in influencing *A. lolii* concentrations in perennial ryegrass. Studies have indicated that *A. lolii* hyphal counts are directly related to changes in temperature (20, 38), although there is evidence suggesting that high temperatures may be associated with a reduction in endophyte growth (13, 14). There is also evidence suggesting that increased rainfall enhances the growth of *A. lolii* (24), while moisture stress decreases the growth of the endophyte (20).

The results of the present study indicate that *A. lolii* concentrations in both the basal and regrowth components of the ryegrass increased and were at their highest during the period of plant reproductive development (September to January) (Fig. 1). Since the transmission of *A. lolii* is dependent upon the successful completion of the sexual reproductive cycle of the host grass, proliferation of *A. lolii* during flowering may be advantageous to the endophyte, as it could be expected to increase the probability of *A. lolii* successfully infecting the developing ryegrass embryo and therefore of being successfully disseminated. This line of reasoning suggests that, in addition to the temperature and rainfall effects discussed previously, one or more of the factors influencing or resulting from host plant reproductive development (i.e., photoperiod, plant hormone balance, plant nutrient status, plant cell structure, or plant growth rate) may also be important in influencing *A. lolii* concentrations. However, it is difficult to draw firm conclusions since the compositions of both the basal and regrowth components were constantly changing as a result of tiller age (i.e., dead leaf content), plant growth, and plant reproductive development. For example, during the reproductive phase of the plant (from approximately September to February), the basal component contained nodal and internodal tissues (as well as leaf sheath and leaf blade, which were present all year round), while the regrowth component, in addition to leaf blade which was present throughout the year, contained nodal and internodal tissue as well as developing inflorescences. Also, as a result of differential plant growth rates, the height and the amount of regrowth material harvested in midwinter (August) was considerably less than that harvested in spring (October) or autumn (April).

It is also difficult to compare results from basal and regrowth components with each other. In addition to the differences in tissue composition mentioned earlier, much of the regrowth component would have been considerably younger than the basal component at any one time. However, the fact that *A. lolii* concentrations in the regrowth component were lower than those in the basal component over the autumn-winter period (March to September 1992) (Fig. 1) is not surprising, since most of the material in the regrowth component during this period consisted of leaf blade, which is known to contain lower *A. lolii* concentrations than leaf sheath material (4, 36, 39, 40, 42).

The seasonal changes in mean lolitrem B concentrations recorded in the present study (1991 to 1992) (Fig. 3) were similar to those reported in a study conducted in 1990 (8), with concentrations peaking in summer and early autumn (January to April) on both occasions. These results are also in general agreement with those of the pasture-based studies of Prestidge and Gallagher (45) and di Menna et al. (19). Thus, as was observed with *A. lolii*, lolitrem B concentrations change according to the time of year, indicating that environmental factors which also change with time of year may be important influences. However, under controlled conditions, Barker et al. (9) found that plant water deficit had no effect on the lolitrem B concentration, and to our knowledge, there are no published accounts of the effects of temperature on lolitrem B concentration. If factors such as these are important, they may not act

on lolitrem B concentrations independently. Also, they may act indirectly, by influencing plant and endophyte growth, for example. Plant composition is also likely to be an important factor influencing lolitrem B concentrations, as the highest concentrations recorded in the present and previous studies (8, 19, 45) coincide with the time of year at which the senescent leaf content of the plant is highest and senescent and dead leaf tissues are known to contain the highest concentrations of lolitrem B in the vegetative plant (4, 18, 36). This peak also generally coincides with the time of year at which ryegrass staggers outbreaks are most common (28).

As was apparent with *A. lolii*, lolitrem B concentrations were consistently higher in the basal part of the plant than in the regrowth component (Fig. 3). This distribution pattern of lolitrem B in infected ryegrass has been reported previously (18, 19, 31, 36) and probably explains the observation that serious ryegrass staggers outbreaks usually occur when stock are forced to graze on the basal regions of pastures (28).

As observed for *A. lolii* and lolitrem B, mean peramine concentrations were lowest in winter (Fig. 5). The monthly changes in peramine concentration observed in the current study were similar to those recorded in an earlier investigation using the same plants (8). It is apparent then that, like *A. lolii* and lolitrem B, peramine concentration is influenced by the time of year. Thus, factors similar to those discussed for lolitrem B (i.e., temperature, moisture, plant growth rate, endophyte growth rate, and concentration) may be important in influencing changes in peramine concentration throughout the year. It is interesting that, during winter, mean peramine concentrations were three to four times lower than summer-autumn concentrations. This reduction was small compared with that observed for lolitrem B, which declined by a factor between 22 and 67, depending on the plant component. These results indicate either that peramine was metabolized more slowly than lolitrem B or that the production of peramine was maintained at a more constant level throughout the year than that of lolitrem B.

In contrast to what was observed with *A. lolii* and lolitrem B, mean peramine concentrations were generally higher in the regrowth component than in the basal component of the plant (Fig. 5). This distribution of peramine may seem surprising, since *A. lolii* concentrations were consistently lower in the regrowth than in the basal component (Fig. 1). However, it is possible that peramine was translocated from the basal part of the plant into the regrowth component. It is also possible that, as a result of the plant material (and therefore the endophyte) in the regrowth component being younger than that in the basal component and of the different physical natures of the two plant components, more peramine was synthesized in the regrowth than in the base of the plant. Results of previous reports concerning the vertical distribution of peramine in *A. lolii*-infected ryegrass have differed, although the sampling strategies used were not the same in each study. Tapper et al. (51) found that when cut 8 to 10 cm from the crown, basal portions of *A. lolii*-infected ryegrass plants contained higher concentrations of peramine than the upper portions, whereas Fannin et al. (22) found that the highest concentrations of peramine were present in the leaf blade. Although results from the present investigation indicate that peramine concentrations are highest in the upper part of the plant and that the distribution of peramine between lower and upper parts of the plant is seasonally dependent, it should be noted that the composition of the two components also changed from month to month.

Seasonal fluctuations have been recorded for other endophyte-produced alkaloids. Results from the United States,

New Zealand, and Australia show that ergovaline levels in *Acremonium coenophialum*-infected tall fescue (*Festuca arundinacea* Schreb.) and *A. lolii*-infected perennial ryegrass are lowest in winter (12, 47, 50, 53). A similar seasonal pattern was observed with the loline alkaloids in *A. coenophialum*-infected tall fescue (10, 35). Since this pattern is also similar to that observed for lolitrem B and peramine in the present study, it is probable that many alkaloids produced by the association of an *Acremonium* endophyte with a grass undergo seasonal fluctuations in production and are at the lowest concentrations in winter.

In addition to temporal variations in *A. lolii*, lolitrem B, and peramine concentrations, plant-to-plant variations in the concentration of all three parameters, which were generally consistent (and which, in the case of the two alkaloids, were maintained over 3 years) were also recorded (Fig. 2, 4, and 6). Thus, it is evident that factors other than those related to time of year can also influence *A. lolii*, lolitrem B, and peramine concentrations. Plant-to-plant differences in the levels of *A. lolii*, lolitrem B, peramine, and ergovaline in perennial ryegrass have been noted previously (8, 14, 18, 50). Similar observations concerning relative levels of loline and ergopeptine alkaloids in different *A. coenophialum*-infected tall fescue plants have also been made (1–3, 11, 33, 34). There is evidence indicating that the genetic characteristics of both the endophyte and the grass are important in determining the relative alkaloid levels in a plant (1, 15, 16, 33). Davies et al. (18) also presented evidence suggesting the existence of both endophyte genotype and grass genotype effects and suggested, among other possibilities, that differences in alkaloid content could relate to the vigor of the endophyte growth in the host plant. Although data for individual months were variable, the high degree of correlation between the yearly mean *A. lolii* concentration and the yearly mean lolitrem B and peramine concentrations in individual plants observed in the present study (Fig. 8 and 9) indicates that this was certainly the case in the plants examined. It is therefore not surprising that yearly mean lolitrem B and peramine concentrations for all of the plants were also highly correlated (Fig. 7). Thus, it appears that *A. lolii* concentration is likely to be an important factor in determining lolitrem B and peramine concentrations for individual *A. lolii*-infected perennial ryegrass plants derived from New Zealand pastures. These results therefore indicate that endophyte concentration may be an important consideration for selecting desirable grass-endophyte associations.

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